Committee:
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TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty-Second Street

New York 17, N.Y.

#274

Application for Research Grant

Date: April 30, 1960

1. Name of Investigator: Arthur A. Stein, M.D.

2. Title: Professor of Pathology

3. Institution & Address: Albany Medical College
New Scotland Ave.
Albany, New York

4. Project or Subject: The induction of bronchial epithelial hyperplasia by human cancerous bronchial secretions.

The role of air pollutants in relationship to the incidence of bronchial epithelial hyperplasia as related to the above program.

5. Detailed Plan of Procedure:

See attached material.

6. Budget Plan:

(See page 3)

a.	Salaries	$ 17,800.00
b.	Expendable Supplies	6,100.00
c.	Permanent Equipment	4,500.00
d.	Overhead (15% of a & b)	3,600.00
e.	Other (travel)	1,000.00
	Total	$ 33,000.00

7. Anticipated Duration of Work:

Three years.

8. Facilities and Staff Available:

See attached material for these items.

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

/s/ A. H. Stein, Director of Project
/s/ R. J. ? Asst. Treas.

Application #274

At the Surgical Forum, 45th Clinical Congress, 1959, we reported on the induction of bronchial epithelial hyperplasia by human cancerous bronchial secretions. Two series of preliminary experiments were reported. In the first experiment, bronchial aspirates were obtained at the time of bronchoscopy from two patients with carcinoma of the lung. These patients had tracheostomies and repeated bronchial aspirations were made until 100 ml. of aspirate was collected. This material was then lyophilized, re-suspended in 100 ml. of isotonic saline, and filtered through a Seitz bacterial filter. A similar procedure was followed in 12 patients who were known to have pulmonary emphysema. These patients were bronchoscoped and the bronchial secretions were obtained. It required a total of 12 patient collections to again obtain a 100 ml. volume as the noncancerous control.

Thirty male hamsters about $4\frac{1}{2}$ months old were divided into three equal groups. One group received saline injections; one group received saline with nonneoplastic aspirate; and one group received saline with neoplastic aspirate. The injection program entailed 0.5 ml. subcutaneously twice weekly for 22 weeks. The animals were then sacrificed. The bronchial tree and other organs were carefully dissected out and fixed in formalin. The bronchial tree was embedded en bloc and step sections were made. Histologic observations revealed polyp formations in the bronchi of 7 of the 10 hamsters which had received injections of the neoplastic material. Neither of the control series showed such formations. The remainder of the organs showed no abnormalities.

Second experiment: In the second experiment endobronchial aspirates at the time of bronchoscopy were obtained from 5 patients with carcinoma of the lung and 4 with pulmonary emphysema. Each sample consisted of approximately 5 to 10 ml. of aspirated material and each was processed individually, as in experiment 1. The samples were lyophilized, re-suspended in tissue culture medium, and passed through a millipore bacterial filter. A portion was then set aside (frozen) as a noncultured control. Human serum was then added to the remainder and this was divided into two portions, one used as a gross medium for S-3 hela cells, the other for FL-amnion. These cultures were grown for 9-10 days, then removed from their bottles and disrupted ultrasonically. Similar groups were pooled. The only abnormality on short-term tissue culture was observed in the FL-amnion neoplastic cultures which showed some cytopathogenic effect after 3 days. Ninety male hamsters, 5-7 weeks old, were divided into six groups of 15 each. Hela, FL-amnion and noncultured; each in a neoplastic and non-neoplastic series. The injection program was the same as previously but was continued for only 7 weeks. The histologic preparations were identical. Again, the hela neoplastic series yielded a 45 percent of polyp formation, the noncultured neoplastic series, a 51 percent incidence and the amnion cultured neoplastic series, a 70 percent incidence. The correlation between the cytopathogenic effect in FL-amnion and the increased incidence of polyp formation in this series was suggestive of a potentiating effect.

Experiment 3. Currently experiment 2 has been repeated in a large enough series to be statistically valid. Furthermore, at the time of sacrifice the bronchi are washed with saline and aspirated. We will attempt to complete Koch's cycle in order to strengthen our opinion that a factor is truly present in the secretions of human cases of bronchogenic carcinoma which can be transmitted and ultimately recovered.

Proposed study: We feel that there is evidence that in the secretions of patients with bronchogenic carcinoma there is a factor which specifically stimulates bronchial epithelium to undergo hyperplasia, even when this material is injected in a distant site. We propose to evaluate the role of various air pollutants in relationship to the incidence of bronchial epithelial hyperplasia as related to the injection program described above.

Currently we feel that the anatomic knowledge that

a) 90 percent of carcinomas arise in the region of the hilum

b) multiple in-situ lesions occur in association with carcinoma of the lung

c) there is relative freedom of the tracheal epithelium suggesting that a factor is secreted by the epithelium or peribronchial glands and in the physiologic movement of secretions to the hilum where they are progressively concentrated.

The maximum concentration should be topically in the region of the hilum. Subsequent to the cough reflex the material is brought up without direct application to the tracheal mucosa. It may be that the effect of air pollutants is direct only in that they alter the chemical character of the secretion, the volume of the secretion and the concentration.

After concentration we propose to inject a series of mice and hamsters, newborn and weanlings, with and without steroid, with the cell-free extract from bronchial secretions of patients with and without bronchogenic cancer. Half of the animals will be subject to periods of smoking and half will remain as controls. It will be of great interest to see whether the bronchial epithelial irritation associated with air pollutants has any effect quantitatively or qualitatively in the nature of the lesions which we have previously described. At this time I might again indicate that the lesions described in our previous experiments were observed without any inflammatory cell response.

As another type of control experiment we propose to reduce our cell-free preparation to a powder, reconstitute them with saline and then create a spray effect which the experimental animal may inhale. This would give us information in regards to the topical effect of bronchial secretions in relationship to the respiratory epithelium.

Subsequently, we will attempt to fractionate our preparations from patients with bronchogenic carcinoma in order to identify the particular chemical fraction which appears to be responsible for bronchial epithelial changes.

The next step in this program will be to separate chemical fractions in the prepared cell-free materials from controls and patients with bronchogenic carcinoma. Chemically our efforts will be directed towards the separation of proteinaceous, nucleic acid or virus-like material. On the basis of physical properties various fractions could be separated and evaluated in relationship to associated injection experiments.

Budget:

a.	Expendable supplies--		
	Animals	$ 4,000	
	Glassware	1,000	
	Filters	300	
	Chemicals	800	
			$ 6,100
b.	Permanent equipment--		
	Animal cages	500	
	Freeze-dry unit	1,500	
	Bank of smoking machines	1,500	
	Fraction collector	1,000	
			$ 4,500
c.	Other-- Travel to scientific meetings	1,000	1,000
d.	Personnel--		
	Animal care	3,600	
	2 tissue technicians) 1 biochemical technician) @ $4,000	12,000	
	1 part-time secretary	1,200	
	2 part-time medical student assistants	1,000	
			17,800
e.	Overhead -- 15% of a and d	3,600	3,600
		Total	33,000

Facilities and staff available-- My Curriculum Vitae is enclosed. Dr. Michael Vanko (Ph.D), Instructor, and Director of the Clinical Laboratories at the Albany Hospital will be basically in charge of the development of the fractionation and chemical analyses of the prepared material. Joseph V. Landau, Ph.D., Research Associate in Oncology, will be primarily responsible for the preparation of the bronchial secretions. Allan Stranahan, M.D., Associate Professor of Thoracic Surgery, is cooperating by providing us with all the material from patients who are bronchoscoped in his very active department.

Anticipated Duration of Work: The program is planned for a three-year period. The budget for the first year has been outlined on page 5. We feel that in the next two years a budget of $24,000 annually will be necessary.